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Effects of the Theranekron®" an alcoholic extract of the *Tarantula cubensis*" on hematology and serum biochemical properties in horses

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Abstract

Theranekron®is commercially available, alcoholic extract of the *tarantula cubensis*(brown spider).Ten healthy thoroughbred mare racehorses were used at the present study. Blood samples were taken 30 minutes before and 8, 24, 48, 72 and 168h aftersubcutaneous administration of 10ml Theranekron (1mg/48kg or 0.02mg/kgbw) via a jugular catheter. The results of this study showed that sampling time had a significant effects on the amount of PCV, hemoglobin concentration, RBC number, total protein, albumin, glucose, cholesterol, BUN, creatinine, bilirubin, activity of ALT, and ALP (p<0.05) while, had no significant effects on MCV, MCH and MCHC amounts, WBC numbers, fibrinogen concentration, AST, CPK and GGT activities (p>0.05). In conclusion, most of the observed changes in hematological and serum biochemical parameters were statistically and not clinically significant. Thus it seems that administration of Theranekron has no adverse reaction in experimental horses.

Keywords: brown spider, venom, horse, theranekron, *tarantula cubensis*

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Introduction

Venom is a part of defensive or prey capture apparatus of venomous creatures which also assist in chemical digestion (Cousin and Bon, 1999; Honkanen and Golden, 2002; Dounay and Forsyth, 2002). Venom isa complex mixture of various compounds such asproteins, enzymes, ions, biogenic amines, polyamines, cytolytic peptides, and variety of toxins. Most of these materials have been shown to havespecific and diverse pharmacological activities (Li-Smerin and Swartz, 2001; Milne *et al*., 2003). They interfere with important physiological function of cells, result in organ injury, dysfunction or ultimately death (Olivera *et al*., 1984).For example, toxins which target, ion channels and receptors in different cells have been isolated from spiders, marine snails, snakes, scorpions, and some other venomous animals (Fry *et al*., 2005). In addition, several reports indicated thatsome toxinscan affectblood and plasma biochemical parameters (El-Asmar *et al*., 1986; Aguiyi *et al*., 2001; Al-Jammaz, 2003 and Muhammad, 2009).

Moreover, many of these toxins have proven to be invaluable research tools and have provided leads for potential new therapies (Diochot *et al*., 2003). A number of these toxins have already been used in vivo for proof of concept studies. While some of them have pre or clinical proof for pain management, others use to treat diabetes, cancer, multiple sclerosis, and cardiovascular disease (Lewis and Garcia, 2003).

Among the venomous spiders, *tarantula cubensis* is the famous one and many therapeutic effects have been reported for its venom (Stampa, 1986). Theranekron is commercially available as an alcoholic extract of the whole*tarantula cubensis*. In 1977 Mezger described the homeopathic effects of Theranekron (Mezger, 1977). Theranekron® remains active in pharmaceutical compounds for a considerable time. Many therapeutic effects have been described for Theranekron such as; antiphlogistic, demarcative, necrotizing action and wound healing (Stampa, 1986, Koch and Stein, 1980; Stampa, 1986; Sardari *et al*., 2007).

Theranekron has been used in cattle, horse, sheep, goat and dog for different purposes. Literature confirmed that Theranekron can be used successfully in cow caseswith necrotic wounds, retained placenta and pododermatitis circumscripta (Koch and Stein, 1980; Stampa, 1986). In dogs Theranekron was used to stop growth of mammary tumors (Koch and Stein, 1980).

The aim of the present study was to evaluate the effects of the Theranekron on hematology and serum biochemical properties in clinically normal horses.

Materials and Methods

Horses

Ten healthy thoroughbred mare racehorses of age 6±1years and weighing, 460±30 kg were used. The horses were housed in stable, fed with a maintenance ration three times per day and had free access to water. Theywalked 30 minutes twice a day during the study period.

Theranekron® alcoholic extract (1:100) of*Tarantulacubensis* in alcoholic solution 1mg/ml, purchased from Richter-Pharma AG, Wels, Austria.

Experimental set-up

Blood samples were taken 30 minutes before and 8, 24, 48, 72 and 168h via a jugular catheter after administration of 10 ml Theranekron subcutaneously, based on the advised dose by manufacturer for a 485 kg horsealmost 0.02mg/kg bw. Blood was collected into ethylenediam-inetetraacetic acid (EDTA) and plane tubes for biochemical and hematological analysis respectively.

Hematological and biochemical analysis

Anti-coagulated blood was used for CBC determination using automated veterinary hematology analyzer (Nihon Kohden, Cell Tac a, MEK 6108, Tokyo, Japan). Differential

leukocyte count was performed microscopically on Giemsa stained blood film using cross sectional method. Plane tubes were centrifuged at 1800g for 10 min followed by removal of serum. Serum was stored at -20 C° until analyzed. The amounts of total serum protein (tp), albumin (alb), urea , creatinine (cre), glucose (glu), cholesterol (chol), total bilirubin (bil), alkaline phosphatase (ALP), creatin kinase (CK), gamma glutamyltransferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by commercial kits (Pars Azmoon, Tehran, Iran) using an autoanalyser (Biotecnica, Targa 3000, Rome, Italy). Control serum (Randox control sera, Antrim, UK) was used for accuracy.

Statistical analysis

Statistical analyses were performed using the SPSS 9 program for windows (SPSS. Inc., Chicago IL, USA). Sampling time effects were examined using ANOVA. All of the analysis was corrected for repeated measurements,time of sampling as fix and horses as random factor were used. In addition paired t-test was used for the comparison of sampling stages with first sampling time. P<0.05 was considered as significant.

Results

The results are presented in tables 1 and 2. Sampling time had a significant effect on PCV amount (p=0.01), there were significant differences between hours 48, 72 and 168 compared with the amount at first sampling time (p<0.05). Sampling time had a significant effects on HB concentration (p=0.01), but there were no significant differences between various sampling time compared with the amount at first sampling time (p>0.05). Sampling time had a significant effect on RBC number (p=0.014), there were significant differences between hours 8, 72, and 168 compared with the amount at first sampling time (p<0.05). Sampling time had no significant effect on MCV (p=0.267) and MCH (p=0.069) value, but there were significant differences in MCH levels between hours 8 and 72 compared with the amount at first sampling time (p<0.05). Although, sampling time had no significant effect on MCHC (p=0.07), but there were significant differences between hours 8 and 72 compared with the amount at first sampling time (p<0.05). Sampling time had no significant effect on WBC numbers (p=0.2), but there were significant differences between hours 72 compared with the amount at first sampling time (p<0.05). Sampling time had no significant effect on neutrohpil (p=0.25), eosinophil (p=0.64), lymphocyte (p=0.49), monocyte (p=0.11) and platelet (p=0.47), there were no significant differences between various sampling time compared with the number at first sampling (p>0.05). Sampling time had a significant effect on TP amounts (p=0.00), there were significant differences between hours 24, 48 and 72 compared with the amount at first sampling time (p<0.05).Sampling time had a significant effect on albumin concentration (p=0.00), but there were no significant differences between various sampling time compared with the amount at first sampling (p>0.05). Sampling time had no significant effect on fibrinogen concentration (p=0.86) and there was no significant difference between various sampling time to compared with the amount at first sampling (p>0.05). Sampling time had significant effect on glucose concentration (p<0.05), there were significant differences between hours 8 compared with the amount at first sampling time (p<0.05). Sampling time had significant effect on cholesterol concentration (p=0.034), but there was no significant difference between sampling time compared with the amount at first sampling (p>0.05). Sampling time had a significant effect on BUN concentration (p=0.00), there were significant differences between hours 48 compared with the amount at first sampling time (p<0.05). Sampling time had a significant effect on creatinin concentration (p=0.00), but there were no significant differences between

various sampling time to compared with the amount at first sampling ($p>0.05$). Sampling time had a significant effect on bilirubin concentration ($p=0.005$), but there were no significant differences between sampling time to compared with the amount at first sampling ($p =>0.05$). Sampling time had no significant effect on AST activity ($p=0.4$), there were no significant differences between sampling time compared with the amount at first sampling ($p>0.05$). Sampling time had no significant effect on CPK activity ($p=0.3$), but there were significant differences between hours 8, 72 and 168 compared with the amount at first sampling time ($p< 0.05$). Sampling time had no significant effect on GGT activity ($p=0.31$), there were no significant differences between sampling time to compare with the amount at first sampling ($p>0.05$). Sampling time had significant effect on GPT activity ($p=0.05$), but there was no significant difference between sampling time compared with the amount at first sampling ($p>0.05$). Sampling time had significant effect on ALP activity ($p=0.03$), but there was no significant difference between sampling time compared with the amount at first sampling ($p>0.05$).

Table 1. Mean±SE of hematological parameters of horses after injection of 10ml Theranekron® subcutaneously (n=10).

	Sampling time					
	0	**8 hours**	**24 hours**	**48 hours**	**72 hours**	**168 hours**
Hematocrit (%)	38.7±0.8	34.1±1.1	34.8±1.4	35.7±0.9*	33.8±0.7*	35.4±0.9*
Hemoglobin (g/dl)	11.97±0.6	9.57±0.6*	9.75±0.6*	10.27±0.5	9.13±0.5*	11.4±0.6
RBC (10^6/µl)	8.3±0.2	7.2±0.2*	7.4±0.4	7.7±0.2	7.4±0.3*	7.6±0.3*
MCV (fl)	46.4±0.8	46.3±0.8	46.4±0.7	46.3±0.8	46.5±0.7	46.4±0.8
MCH (pg)	14.3±0.7	13±0.7*	13.1±0.8	13.4±0.7	12.4±0.8*	14.9±0.8
MCHC (%)	30.9±1.2	28±1.2*	28.1±1.4	28.9±1.4	26.7±1.5*	32.2±1.7
WBC (10^3/µl)	5.56±0.32	5.55±0.38	4.9±0.2	5.3±0.55	4.6±0.24*	5.74±0.41
Neutrophil (10^3/µl)	2.75±0.23	2.63±0.17	2.55±0.21	2.55±0.16	2.30±0.15	2.92±0.19
Lymphocyte (10^3/µl)	2.35±0.18	2.55±0.29	2.07±0.15	2.34±0.41	2.07±0.21	2.48±0.32
Monocyte (10^3/µl)	0.36±0.049	0.36±0.1	0.19±0.04	0.31±0.074	0.17±0.046	0.24±0.062
Eosinophil (10^3/µl)	0.1±0.03	0.09±0.01	0.06±0.02	0.09±0.04	0.07±0.009	0.1±0.04
Platelet (10^3/µl)	159±7.3	172±9	164±9.1	165±5.7	171±5.9	167±6.5

* Significant difference with first sampling time ($p<0.05$).

Table 2. Mean±SE of serum biochemical parameters of horses after injection of 10ml Theranekron® subcutaneously (n=10).

	Sampling time					
	0	**8 hours**	**24 hours**	**48 hours**	**72 hours**	**168 hours**
Total protein (g/dl)	7.06±0.17	7.1±0.18	6.44±0.16*	6.57±0.13*	6.36±0.08*	6.62±0.14
Albumin (g/dl)	3.38±0.09	3.37±0.09	3.13±0.06	3.32±0.07	3.46±0.06	3.48±0.08
Fibrinogen (mg/dl)	288±68	300±69	322±70	322±49	222±55	289±42
Bilirubin (mg/dl)	2.33±0.11	2.12±0.12	1.88±0.08	2.01±0.1	2.09±0.1	2.12±0.15
BUN (mg/dl)	31.75±1.36	29.33±1.16	29.22±1.32	28.8±1.59*	30.32±1.35	31.96±1.57
Creatinine (mg/dl)	1.35±0.04	1.33±0.03	1.34±0.02	1.26±0.02	1.31±0.02	1.33±0.03
Glucose (mg/dl)	105±1.9	100±4*	108±2	108±2	106±3	106±2
Cholesterol (mg/dl)	82.35±4.2	86.5±4	79.5±3	81.1±3.2	78.7±3	80±3
AST (IU/L)	108±10	107±8	115±9	112±9	118±8	121±10
ALT (IU/L)	7±0.9	8.1±0.6	7.7±0.5	7±0.4	7.5±0.4	7.4±0.4
ALP (IU/L)	190.7±23.5	220.3±11.9	209.4±9.4	210±10.4	213.3±9.4	219±10.2
GGT (IU/L)	15.6±1.9	17.8±1	17.1±0.8	17.9±1.1	17.1±0.8	16.1±0.5
CPK (IU/L)	233±30	322±32	296±22	269±20	282±61	255±48

* Significant difference with first sampling time ($p<0.05$).

Discussion

Venom is a great source of biochemical compounds with considerable pharmacological effects.Venomous animals with this property are a potential source for therapeutic investigations. Tarantula cubensis (Cuban tarantula) is a homeopathic remedywith several therapeutic properties. The effect of its alcoholic extract on wound healing (Sardari *et al*., 2007), bovine papillomatosis (Cam *et al*., 2007), and chronic endometritis in dairy cows(Emberg and Sensen, 2007) have reported, but there is no report of its effect on hematological and serum biochemical parameters.

In the present study, Theranekron administration cause significant decrease of RBC parameters during experiment. These decreases reachedthe lowest level at 8 hours post administration and then increased very slowly toward pre-administration level. Generally, this RBC decreasingmight be a manifestation of a condition that increased erythrocyte destruction, erythrocyte loss through hemorrhage, decreased production of red cell or some combination of these conditions. In the current study, the not really significant changes of WBC, granulocytes and monocytes were in contrast by production disorders of bone marrow.

The not-really-significant changes of platelet ruled out production disorders and hemorrhage while the level of bilirubin was in contrast with RBC destruction. According to the significant decrease of the amount of total protein and albumin, it seems the decrease of RBC parameters was probably related to the shift of interstitial fluid to blood and/or sequestration of RBC in spleen.

Da Silva and collogues (2003), studied the effects of brown spider (*Loxosceles intermedia*) venom on hematological parameter of rabbit. They revealed no significant changes in RBC parameters although the number of nucleated red blood cells significantly decreased in bone marrow of experimental rabbits. Futrell,(1992) suggested no evidence of hemolytic anemia in rabbits following spider venom administration.

In our study, the administration of alcoholic extract of tarantula cubensis had no significant effects on the value of WBC, and any type of leukocytes. In contrast, da Silva 2003, reported significant changes in the values of WBC, and neutrophils in blood of rabbits were received brown spider venom. They believed these changes attributed to transient bone marrow depression, influx of netrophils to tissue and tissue necrosis due to the venom. In agreement with our results, no significant changes were reported for eosinophil numbers in rabbits following brown spider envenomation (da Silva *et al*., 2003).

The venom of brown spider is able to promote thrombocytopenia (da Silva *et al*., 2003). They believed this effect could be due to bone marrow depression of megakaryocytes and also extensive consumption of platelets at the site of bite and direct effect of venom. In our study, we did not see any significant changes on platelet number. Morphological changes of human RBC as sphero-echinocytosis caused by red – back spider (*Latrodectusmactans*) venom was reported (Flachsenberger *et al*., 1995). We did not observe such an abnormal morphologic change of RBC in horses following administration of alcoholic extract of tarantula cubensis. Also, fibrinogen concentration was not changed significantly during the current study. This indicated that Theranekrone had not any inflammatory effects in experimental horses.

In the present study, the levels of urea and creatinine significantly decreased up to 48 hours after drug administration and then slowly increased. It seems that increased renal clearance due to the higher glomerular perfusion caused these changes. Similarly, the decreased levels of bilirubin and cholesterol could be attributed to the higher amounts of hepatic uptake and excretion via bile. Absence of significant changes of AST and GGT activity indicated that Theranekron did not have any adverse effects on liver. The significant changes of CK and ALT activity were probably originated from striated muscle

but the exact mechanismwas not clear. Also there was no clear explanation forcause of significant changes of ALP activity; however it mayoriginate from the liver.

In conclusion most of the observed changes in hematological and serum biochemical parameters were statistically and not clinically significant. Thus it seems the administration of alcoholic extract of *Tarantula cubensis* (brown spider) has not any adverse reaction in experimental horses.

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تاثیر ترانکرون، عصاره الکلی ترانتولا کوبنسیس بر هماتولوژی و خواص بیوشیمیائی سرم در اسب

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چکیده

ترانکرون بصورت عصاره الکلی ترانتولا کوبنسیس (عنکبوت قهوه ای) در بازار موجود است. در مطالعه حاضر ده اسب سالم نژاد توروبرد مسابقه ای مورد استفاده قرار گرفت. نمونه های خون ۳۰ دقیقه قبل۸و۲۴، ۴۸، ۷۲و۱۶۸ساعت بعد از تزریق زیر جلدی ۱۰ میلی لیتر (۱میلی گرم برای ۴۸ کیلو گرم وزن بدن یا معادل ۰/۰۲میلی گرم برای هر کیلو گرم وزن بدن) ترانکرون، توسط سوند وداجی اخذ گردید.

نتایج این مطالعه شان داد که زمان نمونه گیری بر مقادیر پی–سی–وی، هموگلوبین، بیلی روبین، تعـداد گلبولهـای قرمـز، مقـدار کلـی پروتئین، آلبومین، گلوکز، کلسترول، بی–یو–ان، کراتنین، فعالیت ای–ال–تی و ای– ال–پی بطور معنی داری تاثیر دارد، در حالیکه هیچ تاثیر معنی داری برمقادیر ام–سی–وی، ام–سی–اچ و تعداد گلبول های سفید، و غلظت فیبرینوژن، ای–اس–تی، سی–پی–ک وفعالیتهـای جـی–جی–تی نداشته است. براین اساس چنین نتیجه گیری می شود که بیشترتغییرات مشاهده شده در پارامترهـای همـاتولوژی و بیوشـیمیایی از نظر آماری و نه کلینیکی معنی دار است. بنابر این بنظر می رسدکه تجویز ترانکرون در اسب های مورد آزمـایش هـیچ واکـنش ناخواسـته و منفی در پی نداشته است.

واژگان کلیدی: عنکبوت قهوه ای ، زهر، اسب، ترانکرون، ترانتولا کوبنسیس